PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/002538	10.03.2005	10.03.2004

International Patent Classification (IPC) or both national classification and IPC
C07K16/00, C12N15/16, C12N5/10, C12N15/62

Applicant
LONZA LTD.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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ATTACHMENT G

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

   see separate sheet

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-29, 32-39, 41, 43-47,49, 54-57
	No:	Claims	30, 31, 40, 42, 50, 51, 52, 53
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-47, 49-57
Industrial applicability (IA)	Yes:	Claims	1-47, 49-57
	No:	Claims	-

2. Citations and explanations

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: SWENNEN DOMINIQUE ET AL: "Secretion of active anti-Ras single-chain Fv antibody by the yeasts Yarrowia lipolytica and Kluyveromyces lactis" MICROBIOLOGY (READING), vol. 148, no. 1, January 2002 (2002-01), pages 41-50, XP002350347 ISSN: 1350-0872.

D2: WONG MICHAEL J ET AL: "Processing of human factor I in COS-1 cells co-transfected with factor I and paired basic amino acid cleaving enzyme (PACE) cDNA" MOLECULAR IMMUNOLOGY, vol. 32, no. 5, 1995, pages 379-387, XP002350310 ISSN: 0161-5890

D3: EP-A-1 099 758 (GENENTECH INC) 16 May 2001 (2001-05-16)

D4: SANTOS A D ET AL: "GENERATION AND CHARACTERIZATION OF A SINGLE GENE-ENCODED SINGLE-CHAIN-TETRAVALENT ANTITUMOR ANTIBODY" CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 5, no. 10, October 1999 (1999-10), pages 3118S-3123S, XP000929841 ISSN: 1078-0432 cited in the application

D5: RIDDER R ET AL: "GENERATION OF RABBIT MONOCLONAL ANTIBODY FRAGMENTS FROM A COMBINATORIAL PHAGE DISPLAY LIBRARY AND THEIR PRODUCTION IN THE YEAST PICHIA PASTORIS" BIO/TECHNOLOGY, NATURE PUBLISHING CO. NEW YORK, US, vol. 13, no. 3, March 1995 (1995-03), pages 255-260, XP002008019.

D6: LEE JEEWON ET AL: "Novel secretion system of recombinant Saccharomyces cerevisiae using an N-terminus residue of human IL-1beta as secretion enhancer" BIOTECHNOLOGY PROGRESS, vol. 15, no. 5, 1999, pages 884-890, XP002211009 ISSN: 8756-7938

Novelty (Article 33(1) and (2) PCT)

1) D1 discloses a single chain antibody anti-p20 ras expressed in yeast using a Kex2 protease

processing sequence that links the scFv to a reporter secretory sequence (page 45, Figures 1,2). Therefore, the subject-matter of claims 30, 31, 40, 42, 50, 51, 52, 53 is not novel.

2) None of the remaining cited prior art discloses a yeast cell with humanised N-glycosylation or CHO cells expressing an immunoglobulin heavy and light chain as fusion polypeptide comprising a cleavage site for endoprotease. Hence, the subject-matter of claims 1-29, 32-39, 41, 43-47, 49, 54-57 appears novel.

Inventive step (Article 33(1) and (3) PCT)

3) D2 discloses the recombinant expression in CHO-K1 or Cos-1 cells of human factor I as a fusion protein whereas the heavy and the light chain is linked by a dibasic linker that can be cleaved by PACE (cDNA co-transfected).Cos and CHO cells contains also endogenous level of furin-like endoprotease that can cleave the proform. The same teaching could be derived from D3 disclosing the expression of prorelaxin in CHO cells using two sites of cleavage for furin-like protein, a dibasic and a tetrabasic site (K-R).

The difference between the closest prior art and the present application is the application of the method of D2 or D3 to the generation of an immunoglobulin, in particular having a heavy and a light chain.

The problem can therefore be seen as the provision of a further application for the method of D2 or D3.

The structure of the human factor I (D2) or prorelaxin (D3) is analogous to the structure of an immunoglobulin in that it is also composed of several chains.
Furthermore, attempts to express immunoglobulin as a single fusion protein linked by a ser-glycine linker is disclosed in D4 and D5.

Therefore, in the light of D2 or D3 in combination with D4 or D5 the person skilled in the art would have tried to construct an antibody as a fusion protein using an endoprotease linker in vertebrate cells. Therefore, the subject-matter of claims 1-19, 24, 43-47, 49, 57 does not involve an inventive step.

4) From D1 the expression of antibodies as a fusion protein as well as the use of a kex2 site in yeast is known, the expression in Pichia is disclosed in D5, whereas D6 uses the system in Saccharomyces cerevisiae. Therefore, it is straightforfard to use the yeast system known for its efficiency and high yield to express an immunoglobulin. It is noted that yeast having humanized N-glycosylation are known. The choice of such a strain is obivious in the antibody field in that one is always seeking for less immunogenic antibodies. Therefore, the subject-matter of claims 25-29, 32-39, 41, 47 does not involve an inventive step.

It is noted that for claims 25-29, 32-39, 41, 47 D1 could also be chosen as closest prior art instead of D2 or D3.

5) Further embodiments of claims 20-23 and 54, 55, 56 would only be considered inventive if the method is novel and inventive.

Re Item VII

Certain defects in the international application

6) Claim 48 is missing.